Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 400–409

Journal of Nutritional Biochemistry

Alpha-linolenic acid increases cholesterol efflux in macrophage-derived foam cells by decreasing stearoyl CoA desaturase 1 expression: evidence for a farnesoid-X-receptor mechanism of action☆

Jun Zhang[a,b], Penny M. Kris-Etherton[a,b], Jerry T. Thompson[c], Daniel B. Hannon[c], Peter J. Gillies[d], John P. Vanden Heuvel[a,b,c,*]

[a]Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA 16802, USA
[b]Center of Excellence in Nutrigenomics, The Pennsylvania State University, University Park, PA 16802, USA
[c]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA 16802, USA
[d]Institute for Food, Nutrition and Health, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA

Received 2 March 2010; received in revised form 19 January 2011; accepted 19 January 2011

Abstract

Increased cholesterol efflux from macrophage-derived foam cells (MDFCs) is an important protective mechanism to decrease lipid load in the atherosclerotic plaque. Dietary alpha-linolenic acid (ALA), an omega-3 polyunsaturated fatty acid (PUFA), decreases circulating cholesterol, but its role in cholesterol efflux has not been extensively studied. Stearoyl CoA desaturase 1 (SCD1) is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids (MUFAs). Endogenous MUFAs are preferentially incorporated into triglycerides, phospholipids and cholesteryl ester, which are abundant in atherosclerotic plaque. This study investigated the mechanisms by which ALA regulated SCD1 and subsequent effect on cholesterol storage and transport in MDFCs. Small interfering RNA (siRNA) also was applied to modify SCD1 expression in foam cells. Alpha-linolenic acid treatment and SCD1 siRNA significantly decreased SCD1 expression in MDFCs. The reduction of SCD1 was accompanied with increased cholesterol efflux and decreased intracellular cholesterol storage within these cells. Alpha-linolenic acid activated the nuclear receptor farnesoid-X-receptor, which in turn increased its target gene small heterodimer partner (SHP) expression, and decreased liver-X-receptor dependent sterol regulatory element binding protein 1c transcription, ultimately resulting in repressed SCD1 expression. In conclusion, repression of SCD1 by ALA favorably increased cholesterol efflux and decreased cholesterol accumulation in foam cells. This may be one mechanism by which dietary omega-3 PUFAs promote atherosclerosis regression.

Keywords: ALA; SCD1; FXR; SREBP1C; Cholesterol efflux; Foam cell

1. Introduction

Alpha-linolenic acid (ALA) is an omega-3 polyunsaturated fatty acid (PUFA) that serves as a precursor to eicosapentaenoic acid and docosahexaenoic acid. Long-chain omega-3 PUFAs are dietary modulators that affect triglyceride and cholesterol metabolism. Numerous studies have shown the benefits of a diet high in omega-3 PUFAs in reducing cardiovascular risk factors [1] mostly due to their inhibition of *de novo* lipogenesis [2]. However, the role of omega-3 PUFAs in cholesterol efflux or reverse cholesterol transport (RCT), another cardiac protective mechanism, is still somewhat controversial [3–9].

As an initial and critical step in RCT, cholesterol efflux from macrophage-derived foam cells (MDFCs) is a multistep process in which free cholesterol (FC) is exported from peripheral tissues and cells. Increased cholesterol efflux from foam cells stabilizes the arterial atherosclerotic plaque and prevents subsequent cardiac events by initiating removal of excessive peripheral cholesterol for transport to the liver and subsequent removal. Multiple proteins affect the rate and amount of cholesterol exported from the MDFCs. Membrane transporters [ATP binding cassette (ABC) transporters and scavenger transporters], mitochondrial enzymes (sterol 27-hydroxylase A1) and apolipoprotein (APOE) all play roles in cholesterol efflux [10,11]. In addition, enzymes involved in lipid storage and oxidation pathways can affect the cholesterol efflux capacity by changing intracellular cholesterol pool size. Extracellular lipid acceptors such as apoA-I or high-density lipoprotein (HDL) also impact the rate at which FC and phospholipids are transported out of the cells.

In addition to lowering circulating total cholesterol and low-density lipoprotein (LDL) cholesterol [12], it is not clear whether and how dietary ALA could affect the cholesterol load in the atherosclerotic plaque. Stearoyl CoA desaturase 1 (SCD1) is an endoplasmic reticulum enzyme that converts saturated fatty acids (SFAs; palmitic

☆ Funding sources: Study was funded by California Walnut Commission and partially funded by the Lester and Audrey Peters ('46) Hogan Scholarship.
* Corresponding author. Tel: +1 814 863 8532; fax: +1 814 863 1696.
E-mail address: jpv2@psu.edu (J.P. Vanden Heuvel).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.01.004

acid and stearic acid) to monounsaturated fatty acids (MUFAs; palmitoleic acid and oleic acid). The endogenous MUFAs produced by SCD1, relative to their dietary counterparts, are preferentially incorporated into cholesteryl esters (CEs), phospholipids and triglycerides, all of which are the lipids abundant in atherosclerotic plaque. In liver and adipose tissue, SCD1 expression is regulated by several transcription factors including sterol regulatory element binding protein 1c (SREBP1C) and the peroxisome proliferator-activated receptors (PPARs) [13]. Much less is known about the regulation of SCD1 activity and its biological role in tissues that do not rely heavily on lipogenesis, such as cholesterol-laden MDFCs, the major microscopic feature of the atherosclerotic plaque.

In addition to SCD1's established role in hepatic MUFA synthesis and subsequent triglycerides production, the hypothesis tested herein was that ALA would affect cholesterol metabolism through regulation of SCD1 in MDFCs. Alpha-linolenic acid treatment significantly increases cholesterol efflux, accompanied with decreased SCD1 expression in MDFCs. By manipulating levels of SCD1 using short hairpin RNA inhibitors, we show that SCD1 plays a role in cholesterol efflux in foam cells without causing a disproportionate and adverse increase in FC and that ALA, as a model omega-3 PUFA, modulates cholesterol efflux by decreasing SCD1. We further investigated a regulatory pathway that SCD1 expression was repressed through inhibition of SREBP1C by the action of a nuclear receptor (NR), farnesoid-X-receptor (FXR). This may have important applications for dietary or pharmaceutical treatment or prevention of cardiovascular disease.

2. Materials and methods

2.1. Chemicals

Human LDL, ALA, methyl-β-cyclodextrin (MBCD), ciprofibrate, TO901317, GW4064 and 8-Br cAMP were purchased from Sigma-Aldrich (St. Louis, MO, USA). GW501516 and 9-*cis* retinoic acid (9-*cis* RA) were purchased from Enzo Life Sciences Inc. (Farmingdale, NY, USA). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT, USA). Geneticin was purchased from Invitrogen (Grand Island, NY, USA). Apolipoprotein A-I and lipid-poor HDL were purchased from Calbiochem (La Jolla, CA, USA). Rabbit polyclonal anti-SCD1 antibody was a kind gift from Dr. Alan R. Tall (Columbia University). Rabbit polyclonal anti-ABCA1 and monoclonal anti-ABCG1 antibodies were purchased from Novus Biologicals Inc. (Littleton, CO, USA) and Abcam Inc. (Cambridge, MA, USA). Rabbit polyclonal anti-small heterodimer partner (SHP), anti-ACTIN antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). [1α,2α (n)-^{3}H] (50 Ci/mmol) cholesterol was purchased from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ, USA). Solvent was evaporated under argon, and ^{3}H cholesterol was dissolved in ethanol as a stock concentration of 1 μCi/μl.

2.2. Preparation of bovine serum albumin conjugated ALA

Alpha-linolenic acid was conjugated to fatty acid-free bovine serum albumin (BSA) based on the method described by Calder et al. [14]. Briefly, ALA was weighed and dissolved in ethanol as a stock concentration of 0.5 M. A total of 32 μl of stock solution was transferred to a brown glass vial and dried under argon while an equal volume of ethanol was dried in another vial as a vehicle control. A total of 132 μl of 0.15 M KOH was added to both vials, vortexed and incubated for 1 h at 70°C under argon. Following the incubation, 2 ml of filter-sterilized BSA (2 mM) in phosphate-buffered saline (PBS) was added to ALA and the vehicle control to achieve a final fatty acid concentration of 8 mM. The pH was adjusted to 7.2 to 7.4. The BSA-conjugated ALA and its BSA control were stored at −20°C under argon until use.

2.3. Cell culture

The RAW 264.7 (*Mus musculus* macrophage) and HEK-293 (*Homo sapiens* epithelial) cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated FBS and antibiotics.

2.4. Oxidized LDL preparation

Low-density lipoprotein at a protein concentration of 200 μg/ml was oxidized with 10 μM $CuSO_4$ at 37°C for 24 h. Oxidized LDL (oxLDL) was concentrated using Amicon Ultra centrifugal filter units (Millipore Corp., Billerica, MA, USA). Excessive copper was removed by dialysis against 0.9% NaCl three times for 24 h at 4°C (Slide-A-Lyzer Mini Dialysis Units; Pierce, Rockford, IL, USA) and was subsequently sealed under argon, stored at 4°C and used within a month. Oxidation of fatty acids and protein components of LDL was demonstrated by analyzing thiobarbituric acid reactive substances (three- to fourfold more production of malondialdehyde) and agarose gel electrophoresis (4–5-mm farther migration). Protein concentration of oxLDL was determined by Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Cholesterol efflux

RAW 264.7 macrophages were seeded in 24-well plates at a density of 2×10^5/well. To induce foam cell formation and label intracellular cholesterol pool equally across samples, cells were loaded with oxLDL (12.5 μg/well) and ^{3}H cholesterol (0.25 μCi/well) in growth medium containing 10% FBS. After 24 h, cells were washed twice with HG-DMEM to remove unlabeled ^{3}H cholesterol and recovered in 10% FBS overnight. Foam cells were then incubated with 1% FBS for 12 h to synchronize the cells and allow cholesterol to be distributed into various intracellular compartments followed by an overnight (16–18 h) ALA treatment in 1% FBS. Following the treatment, medium was collected and centrifuged at 13,200×*g* for 10 min to remove cell debris. Cells were harvested by lysis buffer [5 mM Tris Cl+0.1% sodium dodecyl sulfate (SDS)]. Medium and intracellular tritium count [disintegrations per minute (dpm)] were measured by liquid scintillation counting. ^{3}H cholesterol recovered in the media was calculated as $100\times dpm_{Medium}/(dpm_{Cell}+dpm_{Medium})$ %. During lipid acceptor-induced efflux, foam cells were washed twice following treatment and incubated in HG-DMEM containing 0.2% BSA, along with 10 μg/ml apoA-I, 50 μg/ml HDL protein or 1 mM MBCD for 6, 24 and 6 h, respectively, to induce peak cholesterol efflux.

2.6. Cholesterol assay

RAW 264.7 macrophages were seeded in a six-well plate at a density of 1.5×10^6/well. Foam cell formation, treatment and efflux settings were as described in Cholesterol Efflux. Cells were washed twice with cold PBS. Cholesterol was extracted with 1% Triton X100 in chloroform. Total, free and esterified cholesterol concentrations were determined following the supplier's instructions (Cholesterol Assay Kit; Biovision Inc., Mountain View, CA, USA).

2.7. RNA extraction, reverse transcription, real-time polymerase chain reaction

Cells were lysed and harvested using TriReagent according to the manufacturer's instructions (Sigma; St. Louis, MO, USA). A high-capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA) was used for reverse transcription. Twenty nanograms per microliter of cDNA was amplified by SYBR Green Polymerase Chain Reaction (PCR) Master Mix (Applied Biosystems, Foster City, CA, USA) and detected by ABI 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Primer sequences were listed in Table 1.

Table 1
Oligonucleotides used in quantitative real-time PCR

	Forward (5′→3′)	Reverse (5′→3′)
Abca1	acgctcagaggcttcttctgta	caggaccttgtgcatgtcctt
Abcg1	tcctgctcttctccggattc	ggtaggctgggatggtgtca
Cd36	atcaagctccttggcatggta	tcaccactccaatcccaagtaag
Apoe	aggaacagacccagcaaatacg	ggcgatgcatgtcttccactat
Scarb1	gaaccgcacagttggtgaga	tgcacgaagggatcgtcatag
Cyp27a1	ggagggcaagtacccaataaga	cggtggtccttccactgatc
Acat	actccatcttgccaggtgtctt	catcctgtcaccaaagcgtaac
Ceh	atggctgcgtgtctgaagatc	gcaacttgtaggccagtgtcaa
Scd1	atcatgccggcccacat	ggtggtcgtgtaagaactggagat
Srebp1c	agcccacaatgccattgaga	tgctgcaagaagcggatgtag
Hmgcr	tgctgccataaactggatcga	cggcttcacaaaccacagtctt
Srebp2	gcgatgagctgactctcgggga	cagggaactctcccacttgattgct
Acc	aactttgtgcccacggtcat	tgctccgcacagattcttcaa
Fas	cctggaacgagaacacgatct	agacgtgtcactcctggacttg
Aco	tgctcagcaggagaaatgga	ggcgtaggtgccaattatctg
Cpt	cattccaggagaatgccagg	ctggcactgcttagggatgtc
Shp	caggcacccttctggtagatct	tgtcaacgtctcccatgatagg

Abca1: ATP binding cassette A1; *Abcg1*: ATP binding cassette G1; *Cd36*: cluster of differentiation 36; *Apoe*: apolipoprotein E; *Scarb1*: scavenger receptor subfamily B type I; *Cyp27a1*: sterol 27-hydroxylase; *Acat*: acyl-CoA: cholesterol acyltransferase; *Ceh*: cholesteryl ester hydrolase; *Scd1*: stearoyl CoA desaturase 1; *Srebp1c*: sterol regulatory element binding protein 1c; *Hmgcr*: 3-hydroxy-3-methyl-glutaryl-CoA reductase; *Srebp2*: sterol regulatory element binding protein 2; *Acc*: acetyl-CoA carboxylase; *Fas*: fatty acid synthase; *Aco*: acyl-CoA oxidase; *Cpt*: carnitine palmitoyltransferase; *Shp*: small heterodimer partner.

2.8. Western blot

RAW 264.7 macrophages were seeded in 15-cm^2 plate at a density of 5×10^6/plate. Macrophages were loaded with 50 μg/ml oxLDL for 24 h, followed by overnight treatment as indicated in the figure legends. After treatment, cells were collected in lysis buffer [0.25 M sucrose, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA and 1 mM DTT] as described by Heinemann and Ozols [15]. For detection of SCD1, lysates were sequentially centrifuged at 800×*g* and 13,200×*g* to remove nuclear and mitochondria fractions. Supernatant was collected and microsomal protein was obtained by centrifuging at 105,000×*g* at 4°C for 2 h, and the pellet was resuspended in lysis buffer. For detection of ABCA1, ABCG1 and SHP, cell lysates were sequentially centrifuged at 800×*g* and 13,200×*g* to remove nuclear and mitochondria fractions. The resulting supernatant was collected for detection of these proteins. Protein concentration was measured by Bio-RAD DC protein assay kit. Total soluble protein was separated on a 12% (SCD1 and SHP) or 6% (ABCA1 and ABCG1) SDS polyacrylamide gel electrophoresis gel and transferred to a PVDF membrane (Immobilon P; Millipore, Bedford, MA, USA). To detect SCD1 and ACTIN, membrane was blocked by 5% nonfat dry milk in TBS+0.2% Tween 20 (TBS$^+$) at 4°C overnight. The membrane was incubated with primary antibody (anti-SCD1 1:1000 or anti-ACTIN 1:500) at room temperature for 2 h. To detect ABCA1, ABCG1 and SHP, membranes were blotted in 5% nonfat dry milk in TBS$^+$ at 4°C overnight and incubated with primary antibodies (anti-ABCA1 1:500, anti-ABCG1 1:500 or anti-SHP 1:200) again at 4°C overnight. Membranes were washed three times with TBS$^+$ and incubated with horseradish peroxidase-linked secondary anti-rabbit antibodies for 1 h at room temperature. Blots were visualized using an ECL plus Western blot detection kit (GE Healthcare Biosciences, Piscataway, NJ, USA).

2.9. Establishment of SCD1 small interfering RNA macrophage stable cell lines

Short hairpin RNA inhibitor [small interfering RNA (siRNA)] sequence targeting mouse *Scd*1 mRNA and scrambled sequences used as a control were designed by OligoEngine workstation 2 software (OligoEngine Inc., Seattle, WA, USA). Three pairs of oligonucleotides containing the siRNA and control sequences were annealed and cloned into pSuper vector following the protocol of OligoEngine Inc. (Seattle, WA, USA). Inserted sequences were confirmed at The Pennsylvania State University Nucleic Acid Facility. RAW 264.7 macrophages were transfected with siRNA and control pSuper plasmids by Lipofectamine according to manufacturer's recommendations (Invitrogen, Grand Island, NY, USA). Positive cells were selected and enriched by culturing in growth medium containing 300 μg/ml Geneticin. Knockdown of *Scd*1 was proven by real-time PCR and Western blot. The sequence of the selected siRNA was 5′-tgaaagaagatattcacga-3′.

2.10. Cell viability test

RAW 264.7 *Scd*1-siRNA and control macrophages were seeded in a 96-well plate at a density of 20,000/well. Foam cells were induced as described in Cholesterol Efflux. After an overnight incubation in reduced serum medium (1% FBS), 100 μl of CellTiter-Glo reagent (Promega, Madison, WI, USA) was added into each well and incubated for 10 min. Fluorescence measurement was performed following manufacturer's instructions.

2.11. Apoptosis measured by flow cytometry

RAW 264.7 macrophages *Scd*1-siRNA and control were seeded in six-well plates at a density of 1.5×10^6/well. Foam cells were induced for a total of 24 h and incubated in reduced serum medium (1% FBS) overnight. Cells were washed twice with cold PBS and resuspended in 1 ml binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$). One hundred microliters of the resuspended solution was incubated with 0.5 μl FITC Annexin V (kind gift from Dr. Robert A. Schlegel, The Pennsylvania State University) for 15 min in the dark. Before measurement, additional 3 μl of propidium iodide was added to each reaction to identify necrotic cells during measurement. Flow cytometry was performed at the Cytometry Facility, The Pennsylvania State University.

2.12. Plasmids, transfection and viral infection

pGL3-m*Srebp* plasmids were provided as kind gifts from Dr. Joseph L. Goldstein (University of Texas Southwestern Medical Center) [16]. The full-length 5′ flanking promoter region of mouse *Srebp1c* (−10 kb) and three truncated length of *Srebp1c* promoter plasmids were used in the experiments. The truncated *Srebp1c* promoters (−368 bp, −278 bp, −170 bp) were ligated with a 3-kb sequence upstream of start codon of exon2 [16]. The plasmids were transfected into HEK293 cells by Lipofectamine with 50 ng cDNA per reaction in a 96-well plate. DNA sequence of FXR ligand binding domain was fused into DNA-binding domain of pM Gal4 under the control of the SV40 promoter based on the methods described elsewhere [17]. The plasmid (45 ng per reaction) was co-transfected with pFR, a plasmid which encoded the UAS-firefly luciferase reporter under the control of the Gal4 DNA response element. All reactions were co-transfected with *Renilla* luciferase plasmid (pRL-TK) as internal control. Coding sequence of SHP was amplified by PCR using mouse primary hepatocyte cDNA as a template with primers tailed with *Bam*HI and *Eco*RI restriction sites. It was finally subcloned to retroviral expression plasmid pBABE-neo. HEK293 cells were transfected with 4.6 μg pBABE-m*Shp*, 2.4 μg pCMV-VSV-G-RSV-Rev, 2.4 μg pCAG-HIVgp and 16.5 μl Lipofectamine in 15-cm^2 plates. After 5 h, volume was brought to a total of 10 ml and incubated overnight. The DNA complex was removed the next day, and viruses were expressed by HEK293 cells and secreted in the growth medium for 72 h. The medium was collected, spun, filtered and used to infect RAW 264.7 macrophages with 2 μg/μl Polybrene for 24 h.

2.13. Statistical analyses

Normality of the data was checked by the Anderson–Darling test. General linear model analysis of variance, followed by Tukey's post hoc test, was used to test the difference between treatments (P<.05). The values were expressed as mean±S.E.M. All data analyses were performed by Minitab Version 15 (Minitab Inc., State College, PA, USA), and data were plotted by Prism 5.01 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. ALA increases cholesterol efflux and reduces gene expression in foam cells

Acceptor-induced (apoA-I, HDL and MBCD) cholesterol efflux change was examined following ALA treatment to differentiate mechanisms of efflux (ABCA1-apoA-I, ABCG1-HDL interaction and nonspecific efflux induction). Labeled cholesterol efflux was increased by 35%, 25% and 30%, respectively, in the presence of apoA-I, HDL and MBCD compared with BSA control at the peak efflux time (Fig. 1A). The percentage efflux change between BSA- and ALA-treated cells was not different among acceptor- and non-acceptor-induced efflux. In the absence of any lipid acceptor, ALA (100 μM) significantly increased cholesterol efflux by 33% compared with BSA control (Fig. 1B). This effect was associated with significant reduction of CE, total cholesterol and FC compared with the control (Fig. 1B). Messenger RNAs of genes related to lipid transport (Fig. 1C) and lipogenesis (Fig. 1D) were examined to identify which changes might contribute to the increased cholesterol efflux and decreased intracellular cholesterol levels. Among the tested genes, *Abca1*, *Abcg1* (Fig. 1C) and *Scd1* (Fig. 1D) mRNAs were significantly decreased by ALA treatment in a dose-dependent manner (25–100 μM). Transcripts of other genes were not significantly affected by ALA treatment. ABCA1, ABCG1 and SCD1 protein were further examined. Although *Abca1* and *Abcg1* mRNA levels were altered, ALA treatment did not result in a significant reduction of ABCA1 and ABCG1 protein, the functional forms mediating the cholesterol export (Fig. 1E). In contrast, SCD1 protein was reduced by ALA in a dose-dependent manner (Fig. 1E). After apoA-I- and HDL-induced efflux period (6 and 24 h, respectively), ABCA1 or ABCG1 protein levels were not significantly different between BSA control and ALA-treated cells. However, ALA-treated cells still had less SCD1 protein compared to control cells (Fig. 1F).

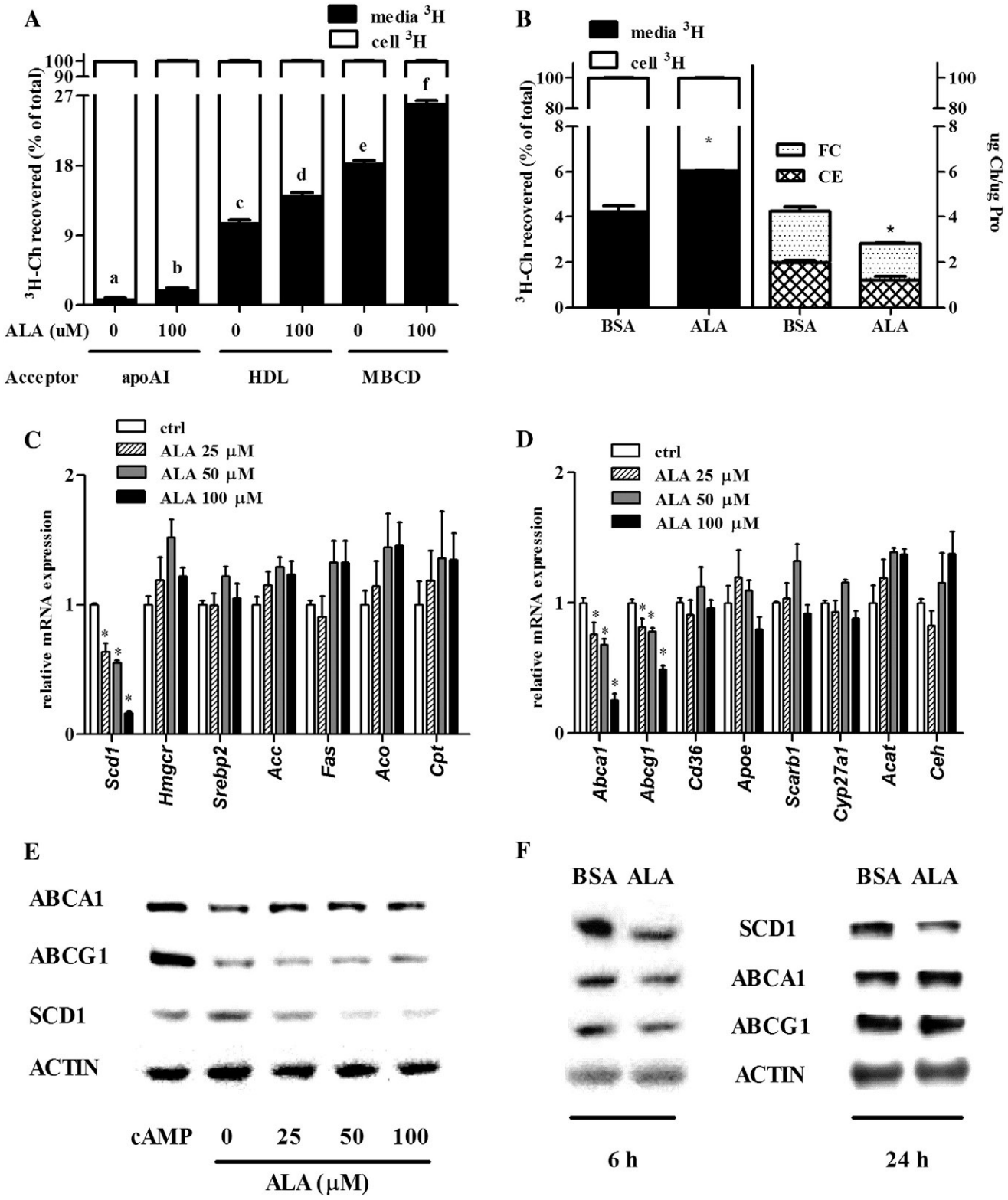
3.2. Repressed SCD1 increases cholesterol export and reduces intracellular cholesterol storage

Alpha-linolenic acid treatment significantly decreased SCD1 expression in foam cells. To delineate the role of SCD1 in ALA-augmented cholesterol efflux, siRNA targeting this transcript was introduced into RAW 264.7 macrophages. Upon siRNA expression, *Scd1* mRNA and protein levels were significantly affected in the appropriate manner, while *Abca1*, *Abcg1* mRNA or protein was not significantly changed (Fig. 2A). Relative to control cells, *Scd1*-siRNA cells had a significantly higher level (36%) of cholesterol efflux in the absence of lipid acceptor (Fig. 2A). In addition, *Scd1*-siRNA cells had a significantly higher level of cholesterol efflux (31%, 38% and 35%) induced by apoA-I, HDL and MBCD compared with the respective control cells (Fig. 2B). The increase of efflux in *SCD*1-siRNA cells was not significantly different in the absence (36%) or the presence (31% to 38%) of lipid acceptors. Accompanied with an increase in cholesterol

efflux, decreasing *Scd1* expression via siRNA significantly decreased the intracellular cholesterol concentration compared with the control cells (Table 2). One concern in modulating cholesterol metabolism is the potential toxicity caused by intracellular accumulation of FC relative to CE. The ratio of FC/CE was not significantly different between *Scd1*-siRNA and control cells during any type of efflux (Table 2). No difference in cell viability or the number of cells undergoing apoptosis was observed between control cells and siRNA stable cells (data not shown).

3.3. ALA reduces SCD1 expression through FXR action

To affect gene expression, certain nutrients, in particular dietary fatty acids, modulate the activity of NRs. Foam cells were treated



with different specific agonists of NRs to investigate the mechanisms by which ALA regulates *Scd1* expression. Among the tested NR activators, GW4064, a specific potent agonist of FXR, significantly reduced *Scd1* mRNA, while TO901317 and 9-*cis* RA, agonists of liver-X-receptor (LXR) and retinoid-X-receptor (RXR), increased *Scd1* expression (Fig. 3A). Ciprofibrate (PPARα agonist), GW501516 (PPARβ/δ agonist), rosiglitazone (PPARγ agonist) and rifampicin (pregnane-X-receptor; PXR agonist) had no effect on *Scd1* expression. To examine whether ALA could affect cholesterol efflux and SCD1 expression via the action of FXR, a cell-based reporter assay was utilized. Alpha-linolenic acid (25–100 μM) significantly increased ligand-dependent *Fxr* activity in a dose-dependent manner (Fig. 3B). Small heterodimer partner (SHP), a target gene of FXR, is a repressor of several NRs including LXR. Whether FXR activation decreases SCD1 through an SHP signaling pathway was examined. Alpha-linolenic acid and GW4064, ligands of FXR, both dose-dependently increased *Shp* expression in foam cells (Fig. 3C). When SHP was overexpressed (Fig. 3D), SCD1 protein was significantly decreased (Fig. 3D).

3.4. FXR decreases Scd1 by repressing Srebp1c expression

As shown in Fig. 3A, while FXR activation led to a reduction of *Scd1*, LXR and RXR increased *Scd1* expression. In foam cells, oxysterols released from oxLDL are natural ligands for LXR. Once activated, LXR forms a heterodimer with RXR and binds to the LXR response element (LXRE) of target genes. SREBP1C is an important regulatory protein in lipogenesis which is regulated in this manner. *Srebp1c* mRNAs were significantly increased when LXR and RXR were activated by their respective ligands, TO901317 and 9-*cis* RA (Fig. 4A). In contrast, FXR activation by GW4064 or ALA resulted in a reduction of *Srebp1c* (Fig. 4A, B). When FXR target gene *Shp* was overexpressed, *Srebp1c* expression also was significantly reduced (Fig. 4B). Importantly, SREBP1C is a regulator of *Scd1* expression [18]; thus, the hypothesis tested is that ALA decreases *Scd1* expression via decreased LXR-dependent transcriptional regulation of *Srebp1c*. The LXR agonist TO901317 significantly increased *Srebp1c* full-length promoter luciferase activity by 3.5-fold compared with that of control (Fig. 4C). In contrast, ALA (100 μM) and GW4064 (0.625–10 μM) significantly decreased *Srebp1c* expression from this plasmid (Fig. 4C). When co-treated with TO901317, ALA (100 μM) and GW4064 (0.625–10 μM) significantly blunted this LXR-dependent *Srebp1c* (full-length promoter) luciferase activation (Fig. 4D). The transcriptional activation of *Srebp1c* by TO901317 required the presence of the LXRE from the *Srebp1c* promoter region (Fig. 4E). When the *Srebp1c*

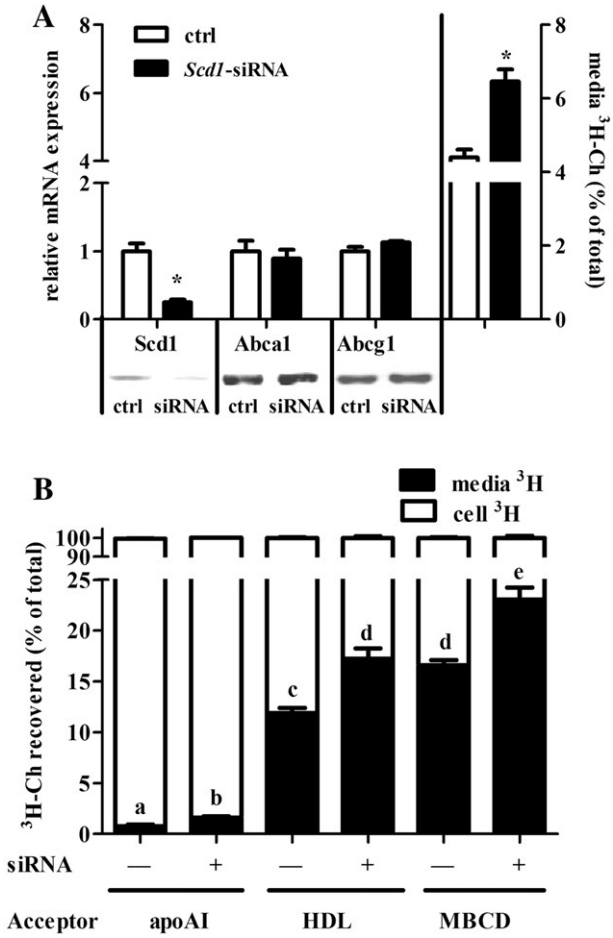


Fig. 2. Cholesterol efflux is increased in *Scd1*-siRNA macrophage stable cell lines. (A) Cholesterol efflux is increased when *Scd1* is knocked down. Messenger RNA and protein changes of *Scd1* and membrane transporters *Abca1* and *Abcg1* in siRNA cells. *A significant difference from control group ($P<.01$). A total of 80 μg protein was loaded on each lane. Results are representative of two independent experiments with triplicate of each group. Foam cells were induced as described in Cholesterol Efflux. Control cells and siRNA cells were washed twice and incubated overnight in 1% FBS+0.2% BSA, and then efflux (non-acceptor-induced efflux) was measured. ^{3}H-Ch recovered in the media of control cells is 4% (of total ^{3}H-Ch; the actual count is 7110 dpm/20,000 cells). *A significant difference from control group ($P<.05$). (B) Cholesterol efflux is increased in siRNA cells in the presence of lipid acceptors. Foam cells were induced as described in Cholesterol Efflux. Cells were incubated overnight in 1% FBS+0.2% BSA. Following incubation, cells were washed twice and incubated in serum-free media with 0.2% BSA together with 10 μg/ml apoA-I, 50 μg/ml HDL protein and 1 mM MBCD for 6, 24 and 6 h to reach respective peak efflux (acceptor-induced efflux). Following respective acceptor-induced efflux period, ^{3}H-Ch recovered in the media of control stable cells stable cells is 1%, 12.5% and 17.6% (of total ^{3}H-Ch) in the presence of apoA-I, HDL and MBCD. The actual counts are 1710 dpm/20,000 cells, 25,170 dpm/20,000 cells and 33,040 dpm/20,000 cells. Bars that do not share common letters differ ($P<.01$). Results are representative of three independent experiments with triplicate of each group.

Fig. 1. Alpha-linolenic acid increases cholesterol efflux and affects gene expression related to lipid metabolism. (A) ^{3}H cholesterol efflux is increased following ALA treatment in foam cells. RAW 264.7 MDFCs were treated with 0.2% BSA or 100 μM ALA +0.2% BSA in 1% FBS media overnight (16–18 h). Cells were washed twice following ALA treatment and incubated in serum free media with 0.2% BSA and either 10 μg/ml apoA-I for 6 h, 50 μg/ml HDL protein for 24 h or 1 mM MBCD for 6 h. Following peak acceptor-induced efflux period (as above; time points of 2, 4, 6, 8, 12 and 24 h were examined), ^{3}H cholesterol recovered in the media of BSA-treated control cells is 1.1%, 10.4% and 18.2%, respectively (of total ^{3}H-Ch) in the presence of apoA-I, HDL and MBCD. The actual counts recovered in the media are 1950 dpm/20,000 cells, 22,430 dpm/20,000 cells and 36,840 dpm/20,000 cells, respectively. Bars that do not share common letters differ. Results are representative of three independent experiments with triplicate of each group. (B) Intracellular cholesterol changes following ALA treatment. RAW 264.7 MDFCs were treated with 0.2% BSA or 100 μM ALA+0.2% BSA overnight. ^{3}H cholesterol recovered in the cell is 96% and 93% (of total ^{3}H-Ch) of BSA- and ALA-treated cells ($P<.05$). The actual counts are 166,070 dpm/20,000 cells and 125,520 dpm/20,000 cells. Quantification of intracellular cholesterol was performed following 16 to 18 h of ALA (100 μM) and BSA control treatment. Cells were washed in cold PBS twice, and cholesterol was extracted with 1% Triton X100 in chloroform. Chloroform was evaporated by vacuum spin, and lipid components were resuspended in 200 μl cholesterol reaction buffer supplied by manufacturer. The measurement was performed following instructions by manufacturer (Biovision Inc., Mountain View, CA, USA). *A significant difference from respective control ($P<.05$). (C) Changes of genes related to cholesterol transport following ALA treatment. Foam cells were treated with ALA for 16 to 18 h. *A significant difference from BSA control ($P<.05$). (D) Changes of genes related to lipid synthesis and oxidation following ALA treatment. Foam cells were treated with ALA for 16 to 18 h. *A significant difference from BSA control ($P<.005$). (E) Protein changes following ALA treatment. A total of 80 μg protein was loaded on each lane. cAMP (0.3 mM) serves as a positive control. Results are representative of three independent experiments. Full names of all abbreviations are as described in Table 1 footnote. (F) ABCA1 and ABCG1 protein changes following efflux period at either 6 h (apo-A-I-induced efflux) or 24 h (HDL-induced efflux). A total of 80 μg protein was loaded on each lane. Cells were treated overnight with either BSA control or ALA (100 μM) before the efflux started. Results of 1B, 1C, 1D and 1F are representative of two independent experiments.

Table 2
Intracellular cholesterol change following cholesterol efflux in *Scd1*-siRNA foam cells

	No inducer		ApoA-I induced		HDL induced		MBCD induced	
	Ctrl	siRNA	Ctrl	siRNA	Ctrl	siRNA	Ctrl	siRNA
TC	3.12±0.37	2.35±0.16*	2.55±0.12	1.95±0.21*	1.31±0.14	1.09±0.06*	1.08±0.09	0.71±0.04*
CE	1.57±0.21	1.16±0.08*	1.2±0.19	0.92±0.07*	0.68±0.07	0.59±0.01*	0.53±0.02	0.38±0.05*
FC	1.55±0.28	1.2±0.31*	1.35±0.26	1.04±0.27*	0.63±0.11	0.5±0.06*	0.55±0.11	0.32±0.01*
FC/CE	0.98±0.22	1.03±0.28	1.13±0.46	1.14±0.43	0.93±0.17	1.18±0.13	1.05±0.33	0.85±0.26

Scd1-siRNA and control cells were seeded in six-well plate at a density of 1.5×10^6/well. Foam cells induction was as described in Cholesterol Efflux. After an overnight (16–18 h) incubation of cells in reduced serum medium (1% FBS), intracellular cholesterol of siRNA and control cells was extracted as "noninducer" group. Cells of acceptor-induced efflux groups were washed twice after overnight incubation, and efflux was induced by 10 μg/ml apoA-I for 6 h, 50 μg/ml HDL protein for 24 h and 1 mM MBCD for 6 h. Cells were then washed in cold PBS twice, and cholesterol was extracted and measured as described in Fig. 1F. Cholesterol content was quantified as μg cholesterol per μg protein. Values were expressed as mean±S.E.M.
* A significant difference from respective control ($P<.05$). Results are representative of two independent experiments with triplicate of each group.

promoter region was truncated but with both LXRE1 and LXRE2 retained, a similar response was observed when co-treating cells with TO901317 and GW4064 or ALA (Fig. 4F). When both LXREs in the *Srebp1c* promoter region were removed, there was no effect of T0901317, ALA or GW4064 on reporter activity (Fig. 4E, F).

4. Discussion

Alpha-linolenic acid, a dietary omega-3 PUFA with demonstrated health benefits, significantly increases cholesterol efflux and reduces cholesterol storage in foam cells. The increased cholesterol efflux

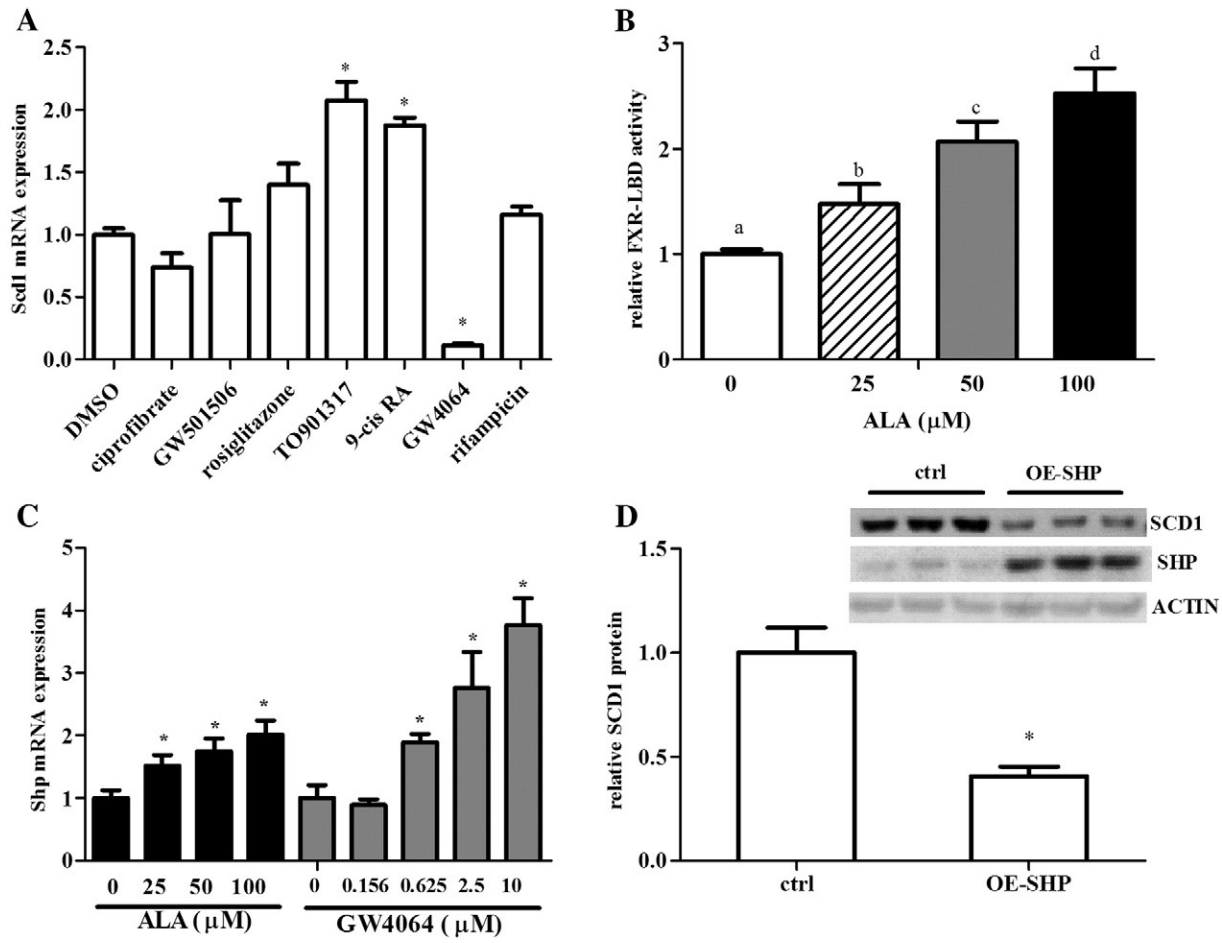


Fig. 3. Alpha-linolenic acid inhibits *Scd1* expression through activating FXR and FXR target gene SHP. (A) *Scd1* mRNA change following different NR agonists treatment. The treatment concentrations were as follows: ciprofibrate 100 μM (PPARα agonist); GW501506 500 nM (PPARβ agonist); rosiglitazone 10 μM (PPARγ agonist); TO901317 5 μM (LXR agonist); 9-*cis* RA 100 nM (RXR agonist); GW4064 10 μM (FXR agonist); rifampicin 25 μM (PXR agonist). Foam cells were treated with different NR agonists for 16–18 h. *A significant difference from DMSO control ($P<.01$). Results are representative of two independent experiments with triplicate of each group. (B) Alpha-linolenic acid (25–100 μM) significantly interacts with FXR ligand binding domain in dual-Luciferase reporter assay. Bars that do not share common letters are statistically different ($P<.001$). Results are representative of three independent experiments with triplicate of each group. (C) Farnesoid-X-receptor target gene *Shp* expression increases following ALA and GW4064 treatment. *A significant difference between treatment and control ($P<.05$). Results are representative of three independent experiments with triplicate of each group. (D) Overexpression of SHP decreases SCD1 protein level. RAW264.7 macrophages were infected with retroviral expression plasmid for 24 h. After overnight recovery, cells were loaded with oxLDL for 24 h and incubated in 1% FBS overnight. A total of 40 μg protein was loaded on each lane with a triplicate of each group. Band intensity was quantified by ImageJ software. Value of SCD1 protein was adjusted by that of ACTIN protein (internal control), and the value of control was normalized as 1. *A significant difference between control (mimic transfection) and SHP overexpression group ($P<.005$). Results are representative of two independent experiments with triplicate of each group.

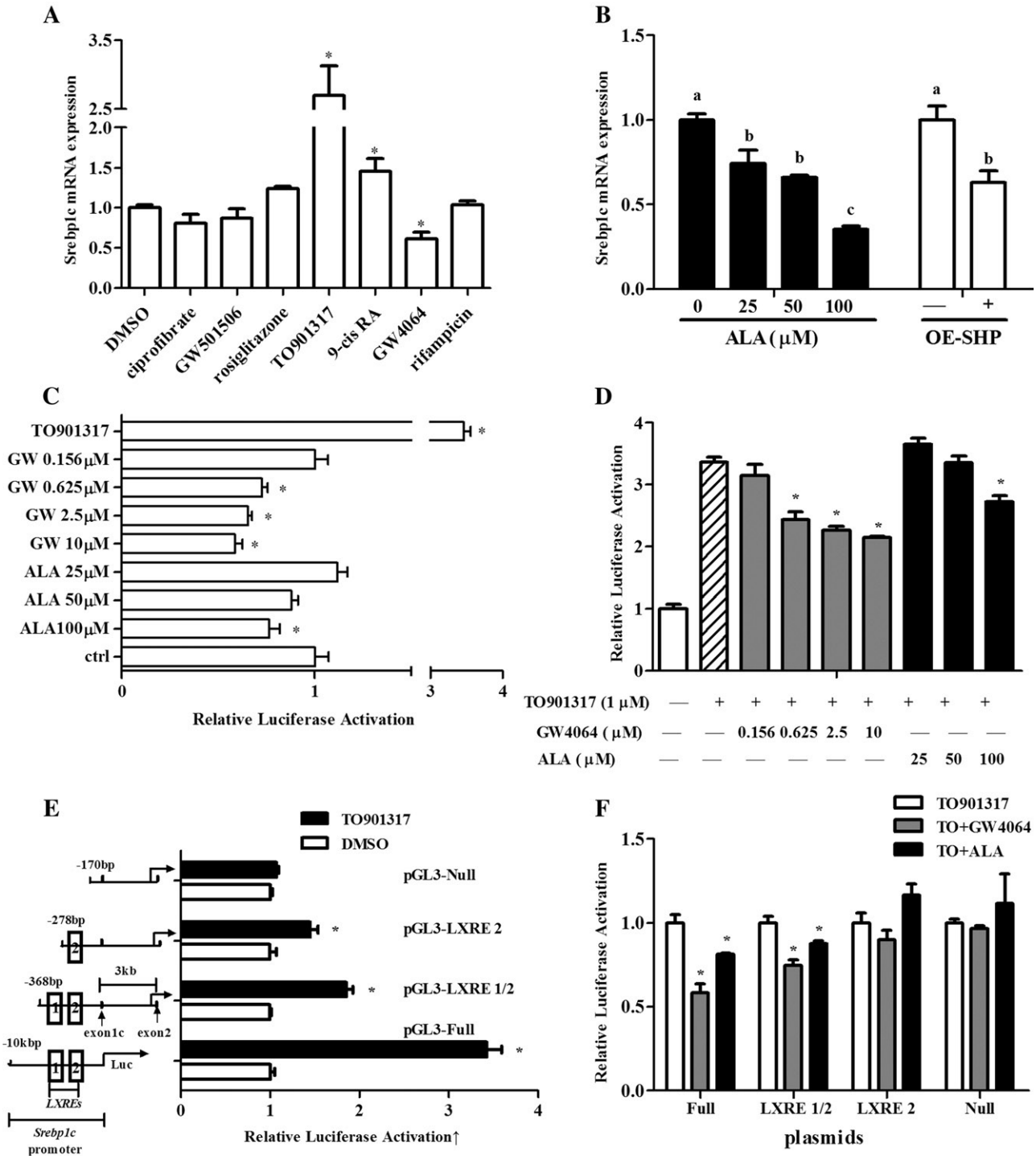


Fig. 4. Farnesoid-X-receptor activation decreases *Scd1* expression by inhibiting *Srebp1c* transcription. (A) *Srebp1c* mRNA change following different NR agonists treatment. Treatment concentration is the same as Fig. 3A. Foam cells were treated with different NR agonists for 16–18 h. *A significant difference from control ($P<.05$). Results are representative of two independent experiments with triplicate of each group. (B) Alpha-linolenic acid and overexpression of SHP (OE-SHP) affected *Srebp1c* transcription. Bars that do not share common letters are statistically different ($P<.05$). (C) Effects of ALA, GW4064 on *Srebp1c* full-length promoter plasmid activity. Liver-X-receptor agonist TO901317 (1 μM) was applied as a positive control to activate full-length *Srebp1c* promoter plasmid activity. *A significant difference between treatment and control ($P<.05$). (D) Effect of co-treatment of ALA, GW4064 with TO901317 (1 μM) on *Srebp1c* full-length promoter plasmid activity. *A significant difference between co-treatment and TO901317 ($P<.05$). (E) Effects of LXR agonist TO901317 (1 μM) on different lengths of *Srebp* promoter plasmids activities. *A significant difference between DMSO and TO901317 ($P<.005$). (F) Effects of LXR agonist TO901317 (1 μM) and co-treatment of ALA (100 μM) and FXR agonist GW4064 (10 μM) on different lengths of *Srebp1c* promoter plasmid activities. *A significant difference between co-treatment and TO901317 ($P<.05$). All results are representative of two independent experiments with triplicate of each group.

following PUFA, especially omega-3 PUFA, treatment also was observed in several other studies [4,5,19–21]. In our study, the beneficial effect was primarily attributed to the inhibition of SCD1 by ALA. Wang et al. [22] found that overexpression of SCD1 resulted in a 71% increase in the oleate to stearate ratio of plasma membrane phospholipids, which was correlated with a decrease in the cholesterol-rich region of the membrane [22]. Reduction of SCD1 decreases MUFAs, which are the preferential substrate for CE

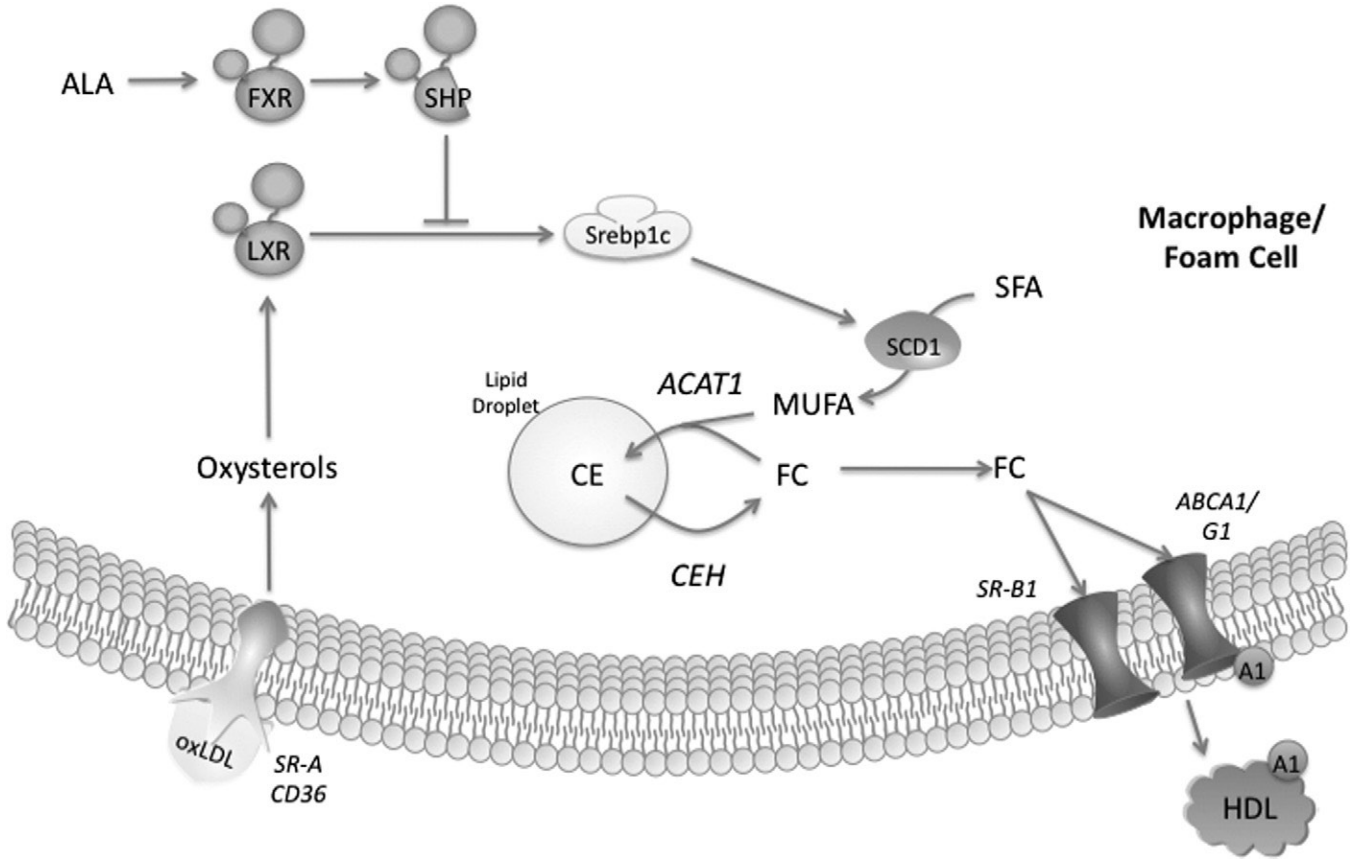


Fig. 5. Schematic regulatory pathway of FXR activation by ALA inhibits SCD1 expression through repressing SREBP1C in RAW 264.7 MDFCs.

synthesis by the enzyme acyl CoA cholesterol acyl transferase (ACAT) [23]. The presence of PUFAs facilitates the FC movement and incorporation to inner leaflet of plasma membrane [24] and subsequent export. Furthermore, previous studies have shown that PUFAs can increase membrane fluidity and permeability [25,26] and alter transbilayer sterol localization, resulting in movement of membrane sterols from cytofacial (inner) to exofacial (outer) leaflets [27]. The cholesterol exported out of the cell is mostly tritium-labeled FC. However, it is possible that the isotope recovered in the media is in the form of oxidized cholesteryl derivatives [10,28], products generated by CYP27A1 (also known as sterol 27-hydroxylase). Although messenger RNA of CYP27A1 (Fig. 1C) was not affected by ALA treatment in our study, quantification of its hydrophilic product, 27-hydroxycholesterol, and other side chain oxysterol products, such as 24S-hydroxycholesterol and cholestenoic acid, will be helpful for understanding the pathways of ALA-induced cholesterol efflux.

In the present study, the increased cholesterol efflux induced by ALA not affecting ABC transporters differs from several other studies where PUFAs decreased efflux by an increased degradation of ABC transporters, possibly through a protein kinase C delta pathway [8,9,29]. In our study, despite reduction in *Abca1* and *Abcg1* mRNA levels, ALA treatment did not dose dependently change ABCA1 and ABCG1 proteins, which mediate cholesterol transport across membranes. This discrepancy could be an effect of the fact that ABC transporters are differently regulated by sterols and fatty acids in macrophages. In our study, foam cells were induced by incubation of oxLDL. Several other studies have shown that cholesterol loading of macrophages markedly increases ABCA1 mRNA abundance and protein levels [30,31], a consequence of activation of LXR by oxysterols [32]. This might lead to undetectable changes of the transporters between different concentrations of ALA treatment in our study. In addition, the extent of acceptor-induced (apoA-I, HDL) efflux between BSA control and ALA treatment was not significantly different from that of nonacceptor or nonspecific acceptor MBCD-induced efflux. Apolipoprotein A-I- or HDL-acceptor-induced cholesterol efflux tests the function of ABCA1- or ABCG1-mediated cholesterol transport [33,34]. Taken together, this ruled out the possibility that ALA induced cholesterol efflux predominantly through increasing membrane ABC transporters. Thus, ALA can alleviate intracellular cholesterol load in foam cells, thereby promoting stabilization of the atherosclerotic plaque.

An important mitigating event in atherosclerosis is RCT, a multistep process resulting in the net movement of cholesterol from peripheral tissues (cholesterol efflux) to the liver via the blood circulation. Multiple steps within this process can potentially be exploited as a drug or nutritional target to increase RCT and subsequently treat or prevent atherosclerosis. SCD1 has been proposed as drug target of obesity [35] and metabolic syndrome [36] due to its central role in lipogenesis. However, the effect of selectively manipulating SCD1 expression in animal atherosclerotic models is unclear, with both positive [37–39] and negative [40,41] outcomes being reported. Some have suggested that accumulation of SFAs following *Scd1* knockdown and subsequent SFA-driven inflammation through Toll-like receptor pathway might be an explanation for the worsened atherosclerotic phenotype [42]. As addressed by MacDonald et al. [40], the relevance of the negative findings such as these to the treatment of human metabolic syndrome is unclear. Our study supported the theory that decreased SCD1 expression in MDFCs would contribute to atherosclerosis regression. When *Scd1* was specifically repressed, cholesterol efflux was significantly increased in the absence of ALA treatment or any lipid acceptors. This result was consistent with a previous study indicating that SCD1 might have an

effect on cholesterol efflux, albeit in nonmacrophage cell lines, possibly related to changes of membrane microdomains and fluidity [22]. The increased cholesterol efflux was accompanied by a significant decrease of all forms of intracellular cholesterol in foam cells. Importantly, the cholesterol storage and transport changes did not result in increased intracellular FC and lipotoxicity [43], which is in stark contrast to the ACAT inhibitors [44].

Another significant contribution of the present study is the elucidation of an NR pathway in the regulation of SCD1 expression. Polyunsaturated fatty acids modulate gene expression via different transcription factors, including the PPARα, β/δ and γ; RXRα, β, and γ; LXRα and β and FXR [45]. In RAW 264.7 macrophages, PPAR and PXR agonists did not reduce *Scd1* expression. Activation of these NRs was typically associated with increased *Scd1* expression [46–55], which could not explain the decreased *Scd1* expression following ALA treatment. In contrast, the specific FXR agonist GW4064 significantly reduced *Scd1* expression similarly to that seen with ALA. Farnesoid-X-receptor has been studied extensively in hepatocytes and enterocytes due to its critical roles in bile acid synthesis, secretion and reabsorption [56]. However, its role in atherosclerosis is somewhat controversial [57–59]. Nonetheless, our results demonstrate that the beneficial effect of ALA on decreased *Scd1* expression is mediated by FXR in RAW 264.7 macrophages (Fig. 5). Oxysterols released from internalized oxLDL serve as ligands of LXR [60], increasing the transcription of *Srebp1c*. This action requires the existence of two LXREs in the *Srebp1c* promoter, while *Srebp2* (effect of SREBP2 on cholesterol homeostasis is reviewed by Horton and colleagues [61]) was not regulated by ALA in foam cells as shown in our study. GW4064 and ALA are incapable of repressing the effect of T0901317 on promoting LXRE2 reporter activity when LXRE1 is deleted from the construct (Fig. 4F). This suggests that the sequence containing only LXRE2 is not sufficient for the action, while the addition of LXRE1 is required to elicit this effect. In this transcriptional network, activation of FXR-SHP blunts the effect of LXR-SREBP1C, which agrees with a previous study showing an LXR repression effect by SHP [62]. However, due to the existence of LXRE in the *Scd1* promoter [63], it also is possible that FXR activation via ALA could directly affect the *Scd1* promoter, independent of *Srebp1c*, to affect its transcription.

In conclusion, these experiments indicate that the omega-3 PUFA ALA increases cholesterol efflux in lipid-laden foam cells in a process that involves decreased SCD1 expression. Manipulating SCD1 in macrophages by pharmacologic or dietary means may improve the removal of oxidized lipids from atherogenic plaques and their subsequent transport to the liver for disposal. More studies are required to test this hypothesis *in vivo* accompanied by end point measurements of relevant physiological responses.

References

[1] U.S. Department of Health and Human Services, Agency for Healthcare Research and Quality. Omega-3 fatty acids, effects on cardiovascular disease. http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=hserta&part=A137995. Accessed on December 18, 2009.

[2] Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 2005;25:317–40.

[3] Nishimoto T, Pellizzon MA, Aihara M, Stylianou IM, Billheimer JT, Rothblat G, et al. Fish oil promotes macrophage reverse cholesterol transport in mice. Arterioscler Thromb Vasc Biol 2009;29:1502–8.

[4] Dusserre E, Pulcini T, Bourdillon MC, Ciavatti M, Berthezene F. Omega-3 fatty acids in smooth muscle cell phospholipids increase membrane cholesterol efflux. Lipids 1995;30:35–41.

[5] Marmillot P, Rao MN, Liu QH, Chirtel SJ, Lakshman MR. Effect of dietary omega-3 fatty acids and chronic ethanol consumption on reverse cholesterol transport in rats. Metabolism 2000;49:508–12.

[6] Gillotte KL, Lund-Katz S, de la Llera-Moya M, Parks JS, Rudel LL, Rothblat GH, et al. Dietary modification of high density lipoprotein phospholipid and influence on cellular cholesterol efflux. J Lipid Res 1998;39:2065–75.

[7] Kilsdonk EP, Dorsman AN, van Gent T, van Tol A. Effect of phospholipid fatty acid composition of endothelial cells on cholesterol efflux rates. J Lipid Res 1992;33:1373–82.

[8] Murthy S, Born E, Mathur SN, Field FJ. Liver-X-receptor-mediated increase in ATP-binding cassette transporter A1 expression is attenuated by fatty acids in CaCo-2 cells: effect on cholesterol efflux to high-density lipoprotein. Biochem J 2004;377:545–52.

[9] Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. J Biol Chem 2002;277:5692–7.

[10] Schmitz G, Langmann T. Transcriptional regulatory networks in lipid metabolism control ABCA1 expression. Biochim Biophys Acta 2005;1735:1–19.

[11] Yancey PG, Bortnick AE, Kellner-Weibel G, de la Llera-Moya M, Phillips MC, Rothblat GH. Importance of different pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 2003;23:712–9.

[12] Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr 2004;134:2991–7.

[13] Ntambi JM, Miyazaki M. Recent insights into stearoyl-CoA desaturase-1. Curr Opin Lipidol 2003;14:255–61.

[14] Calder PC, Bond JA, Harvey DJ, Gordon S, Newsholme EA. Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. Biochem J 1990;269:807–14.

[15] Heinemann FS, Ozols J. Degradation of stearoyl-coenzyme A desaturase: endoproteolytic cleavage by an integral membrane protease. Mol Biol Cell 1998;9:3445–53.

[16] Chen G, Liang G, Ou J, Goldstein JL, Brown MS. Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc Natl Acad Sci U S A 2004;101:11245–50.

[17] Vanden Heuvel JP, Thompson JT, Frame SR, Gillies PJ. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. Toxicol Sci 2006;92:476–89.

[18] Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res 1999;40:1549–58.

[19] Morisaki N, Kanzaki T, Fujiyama Y, Osawa I, Shirai K, Matsuoka N, et al. Metabolism of n-3 polyunsaturated fatty acids and modification of phospholipids in cultured rabbit aortic smooth muscle cells. J Lipid Res 1985;26:930–9.

[20] Pal S, Davis PJ. N-3 polyunsaturated fatty acids enhance cholesterol efflux from human fibroblasts in culture. Biochem Biophys Res Commun 1990;173:566–70.

[21] Mathur SN, Watt KR, Field FJ. Regulation of intestinal NPC1L1 expression by dietary fish oil and docosahexaenoic acid. J Lipid Res 2007;48:395–404.

[22] Sun Y, Hao M, Luo Y, Liang CP, Silver DL, Cheng C, et al. Stearoyl-CoA desaturase inhibits ATP-binding cassette transporter A1-mediated cholesterol efflux and modulates membrane domain structure. J Biol Chem 2003;278:5813–20.

[23] Rudel LL, Shelness GS. Cholesterol esters and atherosclerosis-a game of ACAT and mouse. Nat Med 2000;6:1313–4.

[24] Knapp HR, Hullin F, Salem Jr N. Asymmetric incorporation of dietary n-3 fatty acids into membrane aminophospholipids of human erythrocytes. J Lipid Res 1994;35:1283–91.

[25] Spector AA, Yorek MA. Membrane lipid composition and cellular function. J Lipid Res 1985;26:1015–35.

[26] Wassall SR, Brzustowicz MR, Shaikh SR, Cherezov V, Caffrey M, Stillwell W. Order from disorder, corralling cholesterol with chaotic lipids. The role of polyunsaturated lipids in membrane raft formation. Chem Phys Lipids 2004;132:79–88.

[27] Sweet WD, Schroeder F. Polyunsaturated fatty acids alter sterol transbilayer domains in LM fibroblast plasma membrane. FEBS Lett 1988;229:188–92.

[28] Bjorkhem I, Diczfalusy U, Lutjohann D. Removal of cholesterol from extrahepatic sources by oxidative mechanisms. Curr Opin Lipidol 1999;10:161–5.

[29] Wang Y, Oram JF. Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a protein kinase C delta pathway. J Lipid Res 2007;48:1062–8.

[30] Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, Porter JG, et al. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J Clin Invest 1999;104:R25–31.

[31] Langmann T, Klucken J, Reil M, Liebisch G, Luciani MF, Chimini G, et al. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochem Biophys Res Commun 1999;257:29–33.

[32] Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. J Biol Chem 2000;275:28240–5.

[33] Tall AR, Yvan-Charvet L, Terasaka N, Pagler T, Wang N. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. Cell Metab 2008;7:365–75.

[34] Oram JF, Vaughan AM. ATP-binding cassette cholesterol transporters and cardiovascular disease. Circ Res 2006;99:1031–43.

[35] Dobrzyn A, Ntambi JM. Stearoyl-CoA desaturase as a new drug target for obesity treatment. Obes Rev 2005;6:169–74.

[36] Popeijus HE, Saris WH, Mensink RP. Role of stearoyl-CoA desaturases in obesity and the metabolic syndrome. Int J Obes (Lond) 2008;32:1076–82.

[37] MacDonald ML, Singaraja RR, Bissada N, Ruddle P, Watts R, Karasinska JM, et al. Absence of stearoyl-CoA desaturase-1 ameliorates features of the metabolic syndrome in LDLR-deficient mice. J Lipid Res 2008;49:217–29.
[38] Savransky V, Jun J, Li J, Nanayakkara A, Fonti S, Moser AB, et al. Dyslipidemia and atherosclerosis induced by chronic intermittent hypoxia are attenuated by deficiency of stearoyl coenzyme A desaturase. Circ Res 2008;103:1173–80.
[39] Brown JM, Chung S, Sawyer JK, Degirolamo C, Alger HM, Nguyen TM, et al. Combined therapy of dietary fish oil and stearoyl-CoA desaturase 1 inhibition prevents the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 2010;30:24–30.
[40] MacDonald ML, van Eck M, Hildebrand RB, Wong BW, Bissada N, Ruddle P, et al. Despite antiatherogenic metabolic characteristics, SCD1-deficient mice have increased inflammation and atherosclerosis. Arterioscler Thromb Vasc Biol 2009;29:341–7.
[41] Brown JM, Chung S, Sawyer JK, Degirolamo C, Alger HM, Nguyen T, et al. Inhibition of stearoyl-coenzyme A desaturase 1 dissociates insulin resistance and obesity from atherosclerosis. Circulation 2008;118:1467–75.
[42] Fessler MB, Rudel LL, Brown JM. Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome. Curr Opin Lipidol 2009;20:379–85.
[43] Warner GJ, Stoudt G, Bamberger M, Johnson WJ, Rothblat GH. Cell toxicity induced by inhibition of acyl coenzyme A:cholesterol acyltransferase and accumulation of unesterified cholesterol. J Biol Chem 1995;270:5772–8.
[44] Meuwese MC, de Groot E, Duivenvoorden R, Trip MD, Ose L, Maritz FJ, et al. ACAT inhibition and progression of carotid atherosclerosis in patients with familial hypercholesterolemia: the CAPTIVATE randomized trial. JAMA 2009;301:1131–9.
[45] Vanden Heuvel JP. Diet, fatty acids, and regulation of genes important for heart disease. Curr Atheroscler Rep 2004;6:432–40.
[46] Miller CW, Ntambi JM. Peroxisome proliferators induce mouse liver stearoyl-CoA desaturase 1 gene expression. Proc Natl Acad Sci U S A 1996;93:9443–8.
[47] Kim YC, Gomez FE, Fox BG, Ntambi JM. Differential regulation of the stearoyl-CoA desaturase genes by thiazolidinediones in 3T3-L1 adipocytes. J Lipid Res 2000;41:1310–6.
[48] Montanaro MA, Lombardo YB, Gonzalez MS, Bernasconi AM, Chicco A, Rimoldi OJ, et al. Effect of troglitazone on the desaturases in a rat model of insulin-resistance induced by a sucrose-rich diet. Prostaglandins Leukot Essent Fatty Acids 2005;72:241–50.
[49] Yao-Borengasser A, Rassouli N, Varma V, Bodles AM, Rasouli N, Unal R, et al. Stearoyl-coenzyme A desaturase 1 gene expression increases after pioglitazone treatment and is associated with peroxisomal proliferator-activated receptor-gamma responsiveness. J Clin Endocrinol Metab 2008;93:4431–9.
[50] Riserus U, Tan GD, Fielding BA, Neville MJ, Currie J, Savage DB, et al. Rosiglitazone increases indexes of stearoyl-CoA desaturase activity in humans: link to insulin sensitization and the role of dominant-negative mutation in peroxisome proliferator-activated receptor-gamma. Diabetes 2005;54:1379–84.
[51] Toyama T, Kudo N, Hibino Y, Mitsumoto A, Nishikawa M, Kawashima Y. Effects of pioglitazone on stearoyl-CoA desaturase in obese Zucker fa/fa rats. J Pharmacol Sci 2007;104:137–45.
[52] Soria A, Gonzalez Mdel C, Vidal H, Herrera E, Bocos C. Triglyceridemia and peroxisome proliferator-activated receptor-alpha expression are not connected in fenofibrate-treated pregnant rats. Mol Cell Biochem 2005;273:97–107.
[53] Hebbachi AM, Knight BL, Wiggins D, Patel DD, Gibbons GF. Peroxisome proliferator-activated receptor alpha deficiency abolishes the response of lipogenic gene expression to re-feeding: restoration of the normal response by activation of liver X receptor alpha. J Biol Chem 2008;283:4866–76.
[54] Montanaro MA, Bernasconi AM, Gonzalez MS, Rimoldi OJ, Brenner RR. Effects of fenofibrate and insulin on the biosynthesis of unsaturated fatty acids in streptozotocin diabetic rats. Prostaglandins Leukot Essent Fatty Acids 2005;73:369–78.
[55] Zhou J, Zhai Y, Mu Y, Gong H, Uppal H, Toma D, et al. A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. J Biol Chem 2006;281:15013–20.
[56] Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov 2008;7:678–93.
[57] Mencarelli A, Renga B, Distrutti E, Fiorucci S. Antiatherosclerotic effect of farnesoid X receptor. Am J Physiol Heart Circ Physiol 2009;296:H272–81.
[58] Guo GL, Santamarina-Fojo S, Akiyama TE, Amar MJ, Paigen BJ, Brewer Jr B, et al. Effects of FXR in foam-cell formation and atherosclerosis development. Biochim Biophys Acta 2006;1761:1401–9.
[59] Zhang Y, Wang X, Vales C, Lee FY, Lee H, Lusis AJ, et al. FXR deficiency causes reduced atherosclerosis in Ldlr−/− mice. Arterioscler Thromb Vasc Biol 2006;26:2316–21.
[60] Torocsik D, Szanto A, Nagy L. Oxysterol signaling links cholesterol metabolism and inflammation via the liver X receptor in macrophages. Mol Aspects Med 2009;30:134–52.
[61] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125–31.
[62] Brendel C, Schoonjans K, Botrugno OA, Treuter E, Auwerx J. The small heterodimer partner interacts with the liver X receptor alpha and represses its transcriptional activity. Mol Endocrinol 2002;16:2065–76.
[63] Chu K, Miyazaki M, Man WC, Ntambi JM, Stearoyl-coenzyme A. desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. Mol Cell Biol 2006;26:6786–98.